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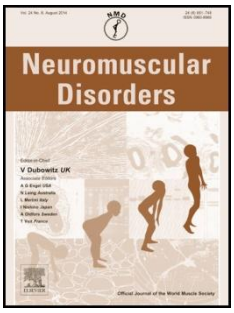

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Parental mosaicism in *RYR1*-related Central Core Disease (CCD)

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Highlights

- First report of parental mosaicism in *RYR1*-related Central Core Disease (CCD).
- Expands the genotypic spectrum of *RYR1*-related disorders.
- Important implications for genetic counselling.

ABSTRACT *(150 words)*

Myopathies due to mutations in the skeletal muscle ryanodine receptor *(RYR1)* gene are amongst the most common non-dystrophic neuromuscular disorders and have been associated with both dominant and recessive inheritance. Several cases with apparently *de novo* dominant inheritance have been reported.

Here we report two siblings with features of Central Core Disease (CCD) born to unaffected parents. Genetic testing revealed a heterozygous dominant *RYR1* c.14582G>A (p. Arg4861His) mutation previously identified in other CCD pedigrees. The variant was absent in blood from the asymptomatic mother but detected at low but variable levels in blood- and saliva-derived DNA from the unaffected father, suggesting that this mutation has arisen as a paternal post-zygotic *de novo* event.

These findings suggest that parental mosaicism should be considered in *RYR1*-related myopathies, and may provide one possible explanation for the marked intergenerational variability seen in some *RYR1* pedigrees.

Key words:

skeletal muscle ryanodine receptor (RYR1) gene; Central Core Disease (CCD); mosaicism

INTRODUCTION

Mutations in *RYR1* encoding the type 1 ryanodine receptor (RyR1), the principal sarcoplasmic reticulum calcium release channel with a crucial role in excitation-contraction (EC) coupling, are the most common cause of non-dystrophic neuromuscular disorders. *RYR1*-related disorders comprise an extremely wide clinical spectrum, ranging from early-onset congenital myopathies of variable severity to induced and episodic phenotypes – malignant hyperthermia (MH) [1], (exertional) rhabdomyolysis (ERM) [2] and periodic paralysis (PP) [3] – in otherwise healthy individuals. Whilst MH and Central Core Disease (CCD), the two disorders originally attributed to disturbed RyR1 function in the 1990s [4], are mainly associated with dominant inheritance of genetic variants localizing to 3 mutational hotspots within the large *RYR1* gene, recessive mutations distributed throughout the entire *RYR1* coding sequence have, more recently, been implicated in subgroups of various congenital myopathies [5], including Multi-minicore Disease (MmD) [6], Centronuclear Myopathy (CNM) [7] and Congenital Fibre Type Disproportion (CFTD) [8]. Apparent *de novo* dominant mutations have also been recognized, often associated with particularly severe manifestations [9].

The genetics of *RYR1*-related myopathies have become increasingly complex: Reflective of the high mutation rate of the large *RYR1* gene, digeny for mutations in *RYR1* and other neuromuscular genes [10, 11] as well as multiple clearly pathogenic *RYR1* variants running independently in the same pedigree [12, 13] have now been repeatedly reported. There is often considerable intra- and interfamilial variability in families with *RYR1*-related myopathies that often remains unaccounted for [5].

Here we report CCD in two siblings due to a heterozygous dominant *RYR1* mutation inherited from an asymptomatic father with somatic mosaicism.

CASE REPORT

Patients

Patient 1: The index case is a 6-year-old girl who presented with bilateral hip dysplasia from birth and subsequent motor developmental delay.

She was delivered from a cephalic position 12 days post term following a normal pregnancy. There were no feeding or respiratory problems. Clinically suspected congenital dislocation of the hips was confirmed on hip ultrasound and treated with open hip reduction and subsequent hip spica cast.

She sat unsupported at 7 months and started crawling at 14 months. Following removal of the hip spica cast at 18 months, she made further slow developmental progress. She was cruising around the furniture at 24 months, took her first independent steps at around 28 months and walked unaided from around three years of age. She is currently able to walk up to 200 metres at a time but tires very easily and falls frequently.

On a recent examination she had mildly myopathic features but a full range of eye movements. She had a positive Gowers' sign and an exaggerated lordosis. There was moderate weakness pronounced axially and in the hip girdle.

An ultrasound of the right thigh showed marked involvement of the quadriceps, with almost complete loss of the femoral bone echo but relative preservation of the rectus femoris, as typically seen in *RYR1*-related myopathies. Previous investigations included normal CK levels (at 65 IU/L) and a normal array CGH. EMG/NCS suggested a myopathic process.

Reviewing the family history, she was the first child of a healthy non-consanguineous Caucasian couple. There was a history of not further defined hip problems in two female cousins of the maternal grandfather. On the paternal side of the family, the paternal grandmother had an undefined problem with her spine that required bracing.

Patient 2: This currently almost 3-year-old boy is the younger brother of Patient 1. He was delivered by normal vaginal delivery at term following an uneventful pregnancy. Apart from gastroesophageal reflux there were no problems in the neonatal period.

He sat unsupported from 7 months, bottom shuffled from 11 months and crawled around the same time, but did not walk independently until18 months of age. At the age of two years and five months, he can crawl up stairs but is still unable to run and falls very frequently.

On a recent examination, there were no overtly myopathic features and the range of eye movements were full. He had a positive Gowers' sign. There was truncal and proximal weakness more pronounced in the hip girdle.

Muscle ultrasound of the right thigh showed features like those in his sister, with marked involvement of the vasti and with relative sparing of the rectus femoris.

Muscle biopsy

A muscle biopsy was obtained from the quadriceps in the older sister at the age of 4 years 4 months and was compatible with a histopathological diagnosis of CCD (Figure 1). There was increased variability in fibre size, increased endomysial connective tissue and scattered internal and central nuclei. There was type 1 fibre uniformity as suggested on oxidative and immunohistochemical stains with antibodies to the slow myosin isoform. There were also few very small "pin prick" fibres staining positive with antibodies to the fetal myosin isoform. On NADH-TR and SDH stains there were well-defined, centrally located cores in most fibres. Cores ran for a significant extent along the fibre axis on longitudinal sections. Cores were to a lesser extent visible on H&E and PAS stains, with a rim of increased glycogen staining on the latter.

Genetic testing

Genetic testing was performed through a custom-made next generation sequencing (NGS) panel covering 36 genes implicated in various congenital myopathies. This revealed a heterozygous *RYR1* c. 14582G>A (p. Arg4861His) sequence variant in the index case, confirmed on direct Sanger sequencing of DNA. The c.14582G>A (p. Arg4861His) variant occurs in a highly conserved amino acid, is absent from the ExAC data set (http://exac.broadinstitute.org/) and has been previously reported in several individuals with CCD and other *RYR1*-related myopathies [5]. NGS in the index case, patient 1, also identified a previously unreported heterozygous *NEB* c.17897C>T (p. Pro5966Leu) variant of uncertain significance. Further cosegregation studies in the family by direct Sanger sequencing using the same primers confirmed the heterozygous *RYR1* c.14582G>A (p. Arg4861His) variant in the similarly affected brother. The *RYR1* variant was absent from the asymptomatic mother but detected at low but variable levels in blood- and saliva-derived DNA from the unaffected father (Figure 2), suggesting that this mutation has arisen as a paternal post-zygotic *de novo* event. The unaffected mother was found to carry the *NEB* c.17897C>T (p. Pro5966Leu) variant of uncertain significance which is unlikely to be contributory to the phenotype in the affected siblings.

DISCUSSION

Mosaicism, the common occurrence of mutant and wild-type cells in the same tissue, is the result of a postzygotic mutation; depending on the developmental stage where it has occurred, this may affect germline cells, somatic cells, or both [14]. Depending on the tissues affected, as in our case mosaicism may be detected on Sanger sequencing of DNA extracted from lymphocytes or other cells routinely used for genetic testing, but may escape detection

if only the germline is affected, certain tissues are spared or mutant expression levels are too low to be detected on routine testing. Although Sanger sequencing is not a quantitative assay, as in this family it is often possible to conclude mosaicism based on the ratio between the 2 nucleotide traces.

Considering important implications for both genetic counselling and prognosis, parental mosaicism is an important concern in apparently sporadically affected children born to asymptomatic parents without unequivocal mutational evidence for recessive inheritance. The phenotypical expression in the mosaic carrier and the associated recurrence risk in their offspring are highly variable, depending on the tissue affected, mutant to wild-type ratio and tissue-dependent tolerance of the mutant allele.

Parental mosaicism has been reported in a wide range of dominantly inherited human diseases, including subgroups of congenital myopathies due to dominant mutations in *DNM2* [15] and *ACTA1* [16, 17], as well as X-linked recessive myotubular myopathy (XLMTM) [18, 19]. Parental mosaicism has also recently been reported in *COL6*-related myopathies [20, 21], accounting at least partially for the often marked variability seen in some families affected by these disorders.

Previously only suggested in one family consisting of a severely affected child and a mildly affected mother with the recurrent *RYR1* p.Ala4940Thr mutation [22], parental mosaicism has also been documented in rare pedigrees with catecholaminergic polymorphic ventricular tachycardia (CPVT), a familial and potential fatal arrhythmia due to dominant mutations in *RYR2*, the cardiac ryanodine receptor isoform. In one of these families, maternal somatic mosaicism was detected following symptom manifestation in the sibling of a girl considered to be sporadically affected [23], in the other maternal somatic mosaicism was identified on a more extensive genetic screen of a family with 3 typically affected siblings without a preceding family history [24].

The finding of parental mosaicism for a heterozygous dominant *RYR1* mutation has important implications for the approach to *RYR1*-related myopathies and may potentially explain several, currently unaccounted for observations concerning these common neuromuscular disorders. Firstly, marked intergenerational variability has been recognized in *RYR1* pedigrees [5], and, in addition to the presence of multiple mutations or other genetic modifiers, may at least be partially accounted for by somatic mosaicism in some family members, corresponding to recent findings in dominantly inherited *COL6*-related myopathies [20, 21]. Along similar lines, somatic mosaicism in the parental generation may also account for the peculiar observation of apparent "anticipation" between two generations in some CCD pedigrees reported in the pre-molecular era [25]. Secondly, somatic mosaicism may at least in some instances also explain the discordance between genotype and phenotype, in particular a discrepancy between results of genetic testing and the in vitro contracture test (IVCT), that has been reported in some families with *RYR1*-related MH [26]. Lastly, although some *RYR1* mutations may have genuinely occurred *de novo*, apparent *de novo* inheritance of a *RYR1*-related myopathy ought to prompt a careful search for somatic mosaicism which may easily be missed if the parental mutation is not expressed in blood lymphocytes.

In conclusion, this report emphasizes parental mosaicism as a relevant mechanism in *RYR1*-related myopathies that ought to be considered and carefully investigated for, bearing in mind important implications for counselling. Parental mosaicism may also provide one possible explanation for the intergenerational variability observed in some *RYR1* pedigrees, and for cases with presumably recessive RYR1-related myopathies in whom only one RYR1 mutation has been identified.

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Figure 1

Muscle biopsy from the right quadriceps in the index case at 4 years 4 months of age. Sections stained with haematoxylin and eosin (A) show mild myopathic fibre size variation, with a dispersed population of small fibres measuring 10 μm or less (arrows, A). Oxidative stains NADH-TR (B) and COX (C) show well-demarcated large, central or eccentric cores devoid of mitochondria, seen as lack of oxidative activity in a majority of fibre and accompanied by uniformity of staining. Some cores display an enhanced rim of oxidative staining. Immunostaining for slow myosin (D) and fast myosin (E) confirms the overwhelming slow fibre predominance and retained immunoreactivity for slow myosin within cores suggesting that most of them are structured cores. Fetal myosin (arrows, F) labels a dispersed population of mostly very small ("pin prick") fibres measuring less than 5 μm. Many of these very small fibres are also immunoreactive for fast myosin (E). Scale bar: A-F: 100 μm

Figure 2

(A) Pedigree of the family reported in this paper. Black symbols indicate the Central Core Disease (CCD) phenotype, +/- indicates heterozygous mutation carriers, -/- indicates the non-mutation carriers and "mos" indicates the mosaicism. (B) Sequencing analyisis of blood-derived DNA demonstrating heterozygous carrier state of the c. 14582G>A p. (Arg4861His) *RYR1* mutation in the two siblings with CCD (II-1 and II-2, arrows) and somatic mosaicism in the unaffected father (I-1, circle), reflected in a small green peak not present in the unaffected mother (I-2) and a normal control (C). Somatic mosaicism was also identified in saliva-derived paternal DNA (data not shown).

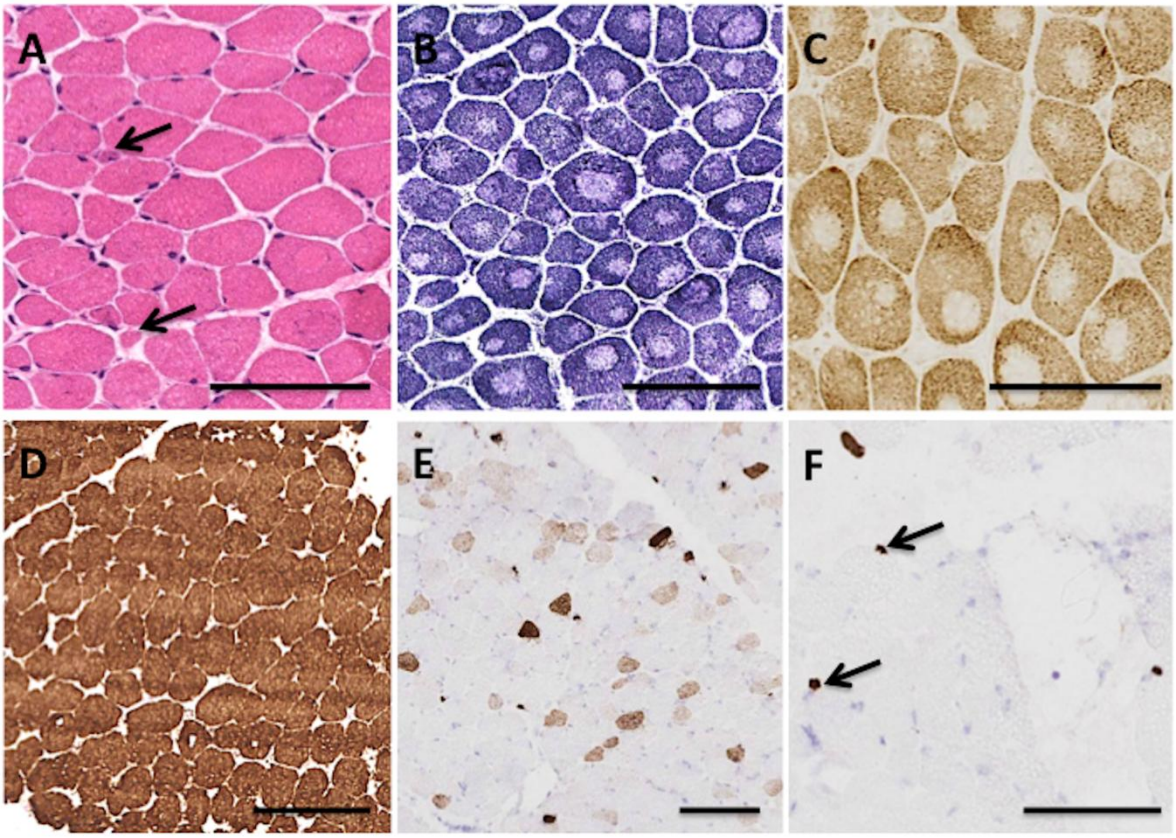


Figure 1

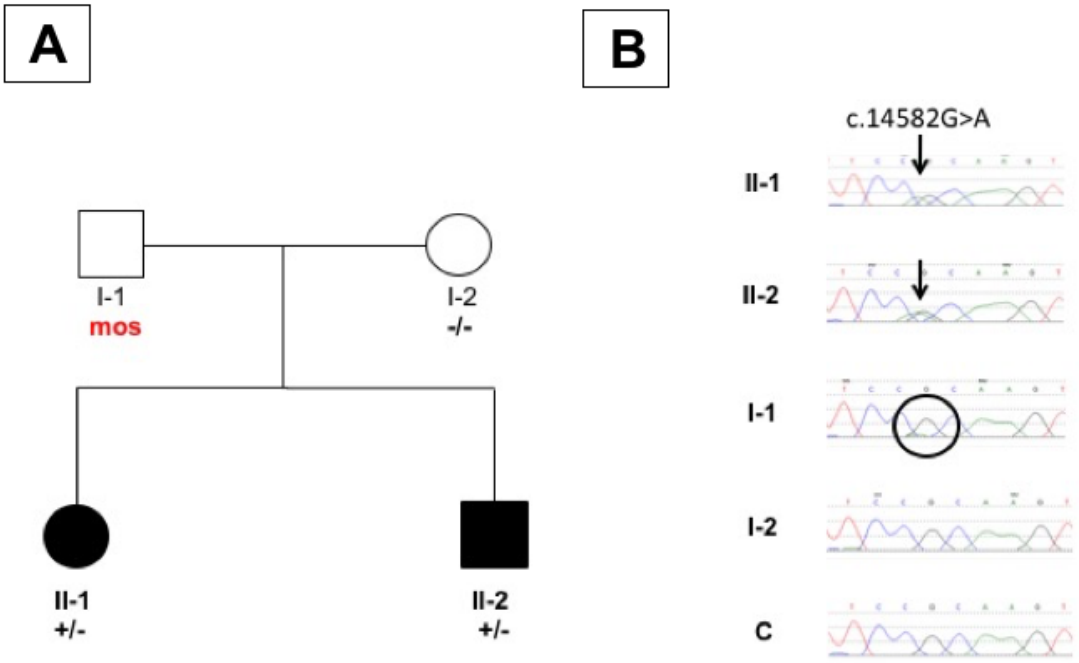
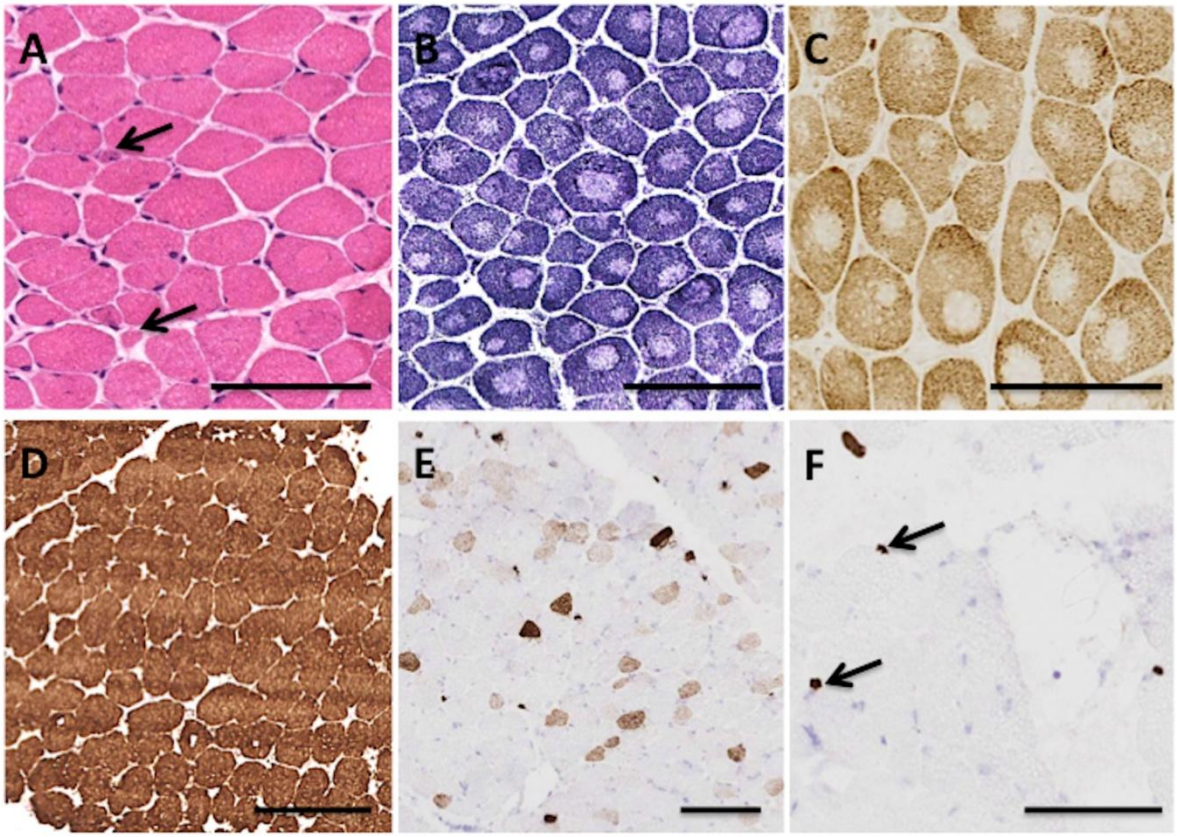


Figure 2



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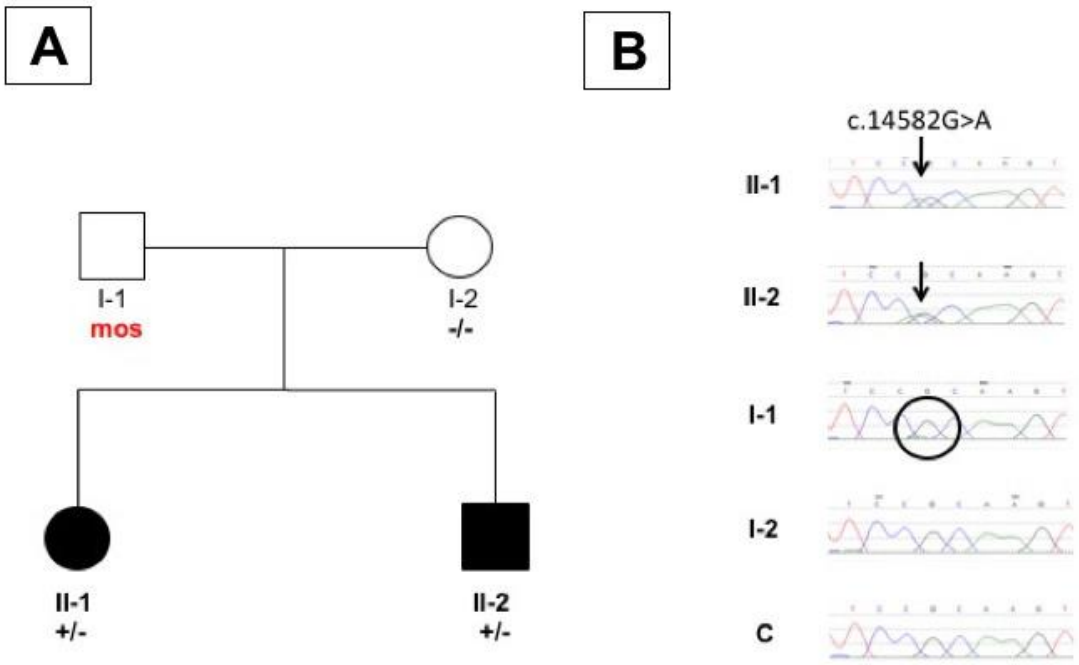

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